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Comparison of the Erythrocyte Sizes of *Carassius gibelio* and *Carassius carassius* Species Living Together in Akgöl (Adapazari/Turkey)

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ABSTRACT

Carassius carassius is one of the fresh water fish species living in Turkey under a natural dissemination. The habitat of this species which is in LC category in the UICN list has recently decreased in parallel with destruction of the ecological conditions of the wetland areas. *C. gibelio* is an alien species for Turkey and the Northern Europe. It is an invader species disseminated in all over the Europe and Turkey by means of its high tolerance ability against the ecological conditions and with the help of a successful reproduction strategy (gynogenetic). One of the natural habitats of *C. carassius* is Akgöl in the city of Adapazari. However, an invader species, *C. gibelio* has also been adapted to the same environment, wherein these two species with close characteristics live together in Akgöl. Blood samples were received from the 4 *C. gibelio* and 3 *C. carassius* specimens using the Laparatomy method wherein 4 blood smear preparations were prepared from each individual. Among the preparations dyed using Gimse method, the photographs of almost 100 erythrocyte cells were taken and computerized. Using the images obtained from the computer, the width and length of the erythrocyte and erythrocyte nucleus were measured by the Spot advanced program which enabled the calculation of the erythrocyte areas, erythrocyte volumes and the area and volumes of the erythrocyte nucleus. The both species with equal chromosome numbers, the erythrocyte and erythrocyte nucleus areas and volumes of *C. gibelio* were found to be larger than *C. carassius*. Particularly the size difference in terms of erythrocyte nucleus area indicates that *C. gibelio* can display a higher power of adaptation by means of the selective gene implication against the ecologically hard conditions.

Key words: *C. carassius, C. gibelio*, erythrocyte size, erythrocyte nucleus size, Akgöl

INTRODUCTION

China is country vast in territory where the complex climatic and geographical conditions have led to evolution of multiplicity of carps. Carps are the major freshwater fish cultured in China and carp culture possesses important economic value in both its culture and productivity. Hence the Chinese Government has paid much attention to economic development and research on carps. By means of such support from the Government a variety of techniques including selection, crossing, gynogenesis, polyploid and introducing new varieties from other regions or countries have been used for improvement of carp. Good varieties and strains carp species have been achieved. There

are 4 *Carassius* genus which constitute economic significance for Chine. They are *C. carassius, C. gibelio, C. cuvieri* (introduced from Japan) and *C. carassius pengzenensis* (Jian *et al*., 2005). Although *C. carassius* and *C. gibelio* genus live in many aqueous conditions within their natural environment in Asia, it is not much possible to see them together since *C. gibelio* is not the natural genus of Turkey.

C. carassius is a fish with Asian origin and particularly lives in the fresh water systems of the northern region of Turkey (Geldiay and Balik, 1988). *C. gibelio* is common in Western Asia, Siberia and the entire Europe (Kottelat, 1997). However, it is not one of natural species in European continent (Flajshans *et al*., 2008). Only its occurrence in Northern Europe may be natural (Kottelat, 1997). *C. gibelio* species was transferred from Asia to Europe in the 17th century (Vetemaa *et al*., 2005). Today, it is considered as an invasive species which spreads in at least 12 European Countries (Ozcan, 2007). *C. gibelio* entered into Turkey through River Maritza over Greece or Bulgaria or it was carried by people (Ozulug *et al*., 2004). Today, all fresh water systems in Turkey are subject to a threat of invasion by *C. gibelio*. This species can adapt to every kind of freshwater system thanks to its high adaptation power and become the dominant species of the environment thanks to its high reproduction power (Arslan and Emiroglu, 2011). *C. gibelio* is well known as a hazardous fish species for native fish communities (Acipinar *et al*., 2006). *C. gibelio* may have a negative effect over native fishes in the environment as they reach the length of reproduction in a very rapid way in the first ages, they lay eggs in a considerable quantity and in a long period and they can reproduce by using eggs of male species of other Cyprinidae species; Furthermore, it is a species that may create substantial water quality problems by speeding up mixing of foreign bodies accumulated in the bottom with water as it mixes the benthos of the fresh water environment it dwells in to find food (Emiroglu *et al*., 2009).

C. carassius species (called as "meç" by the regional fishermen) has been hunted in Akgöl for a long time however *C. gibelio* species was in 1998 for the first time in the lake according to the information obtained from the fishermen. The natural fish fauna of Akgöl also accommodates *Esox lucius* which is from holoarctic origin and the end point of its southern expansion is Turkey. Although *E. lucius* is under LC category according to IUCN, its existence is in danger in Turkey. Since this species is a cold water fish, the increasing water temperature depending on global warming threatens the existence of this fish in Turkey. Moreover, the disrupted ecosystem and the competition of the invasive species with those with a diet based on this fish negatively affect the *E. lucius* populations. It negatively affects *C. gibelio* and *E. lucius* as well as *C. carassius* populations in Akgöl.

The erythrocytes of fish carry a nucleus different from many vertebrates. The most significant function of the erythrocytes within the plasma are carriage of the oxygen dissolved in water to the tissues and discharge of the carbon dioxide formed in the tissues from the body (Timur, 2006). The species of the Carassius genus has 2n = 100 chromosomes in general. The chromosome number of *C. gibelio* and *C. carassius* species is 2n = 100 (FishBase). However, *C. gibelio* can have 3n = 156 chromosomes by means of gynogenesis (Liasko *et al*., 2010). It is an expected situation that the nucleuses carrying the chromosome material of the erythrocytes with identical number of chromosomes and coming from identical genus are close to each other in terms of dimension.

In this study, the erythrocyte sizes of *C. gibelio* and *C. carassius* which joined Akgöl later and created a successful population have been compared.

MATERIALS AND METHODS

In Sakarya city area, Akgöl Lake was formed by descending of the city area from place to place and by ascending of the city area from place to place which was exposed to intense tectonic formations between the late third time and in the early fourth time. The lake was formed after filling of the depressions in the section inclined towards the Black Sea. Akgöl is 8.5 km far from the sea and 2.5 km far from the Sakarya River. While water enters to the lake via the underwater from the west part of the lake, there are not any discharge channels under normal conditions. However, in spring period when the water level is high, the lake gets through to the Sakarya River from time to time via ancient watercourse which remains in the northern end of the lake and reaches to Akkum strait. The domestic waste water from the Gölkent settlement by the lake and the manures used in heavy agricultural activities carried out by the lake reach to the lake via the rain waters. The hunting fish of the lake are *Esox lucius, C. carpio, C. carassius, C. gibelio* and *R. rutilus*. The map of the study area is given in Fig. 1.

In 15 April 2010, blood samples of 4 *C. gibelio* and 3 *C. carassius* sample species caught by Galsama nets from Akgöl were obtained using the Laparatomy method. Smear disseminations were performed on clean lams without allowing coagulation of the blood samples. The blood

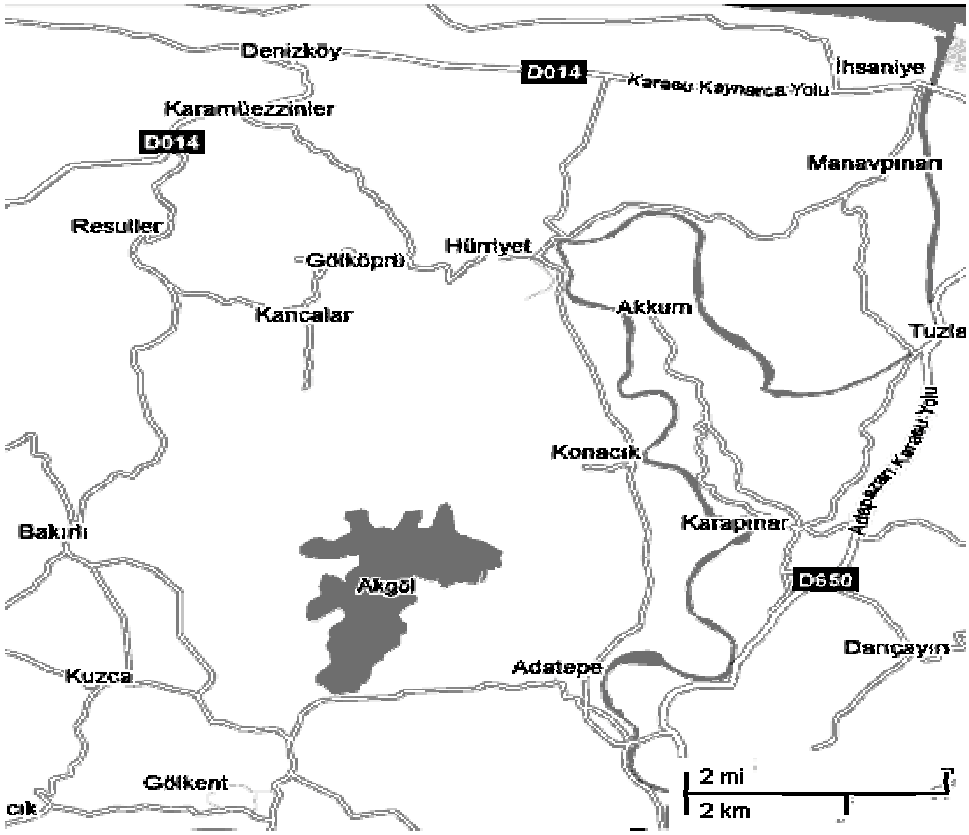

Fig. 1: Akgöl map

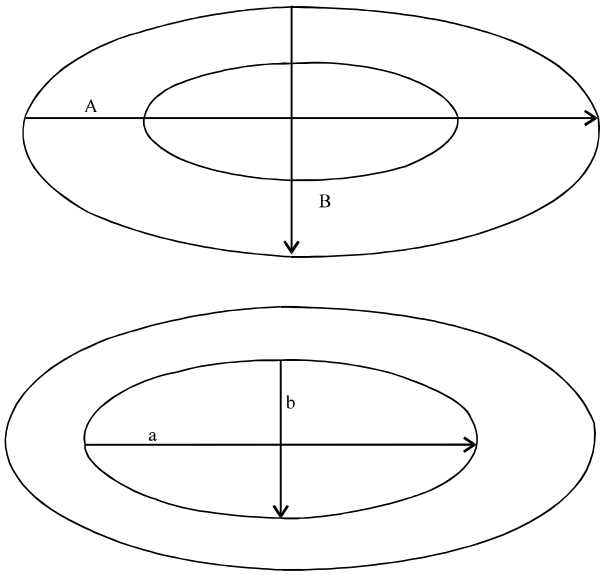

Fig. 2: Measurement chart

preparations dried with air were soaked in Harris haemotoxylin cytologic paint for 3 min and washed after methanol fixation. All the blood preparations were examined under light microscope of Olympus brand CH40 model and photographed with Spot Insgiht brand, 3.2.0. model digital camera and by means of Spot advanced, 4.0.6 version program and then the diameters of about 100 erythrocyte cells and nucleuses from each individual were measured. The areas and diameters of erythrocyte and erythrocyte nucleuses were calculated using the below given formula and the measuring chart presented in Fig. 2.

$$E_{area} = [(AxBxp)/4]\ N_{area} = [(axbxp)/4]$$

$$(E_{volume} = 4/3xpx(A/2)x\ (B/2)^2\ N_{volume} = 4/3xpx(a/2)x(b/2)^2$$

RESULTS

In 15 April 2010, after the blood samples of 4 *C. gibelio* and 3 *C. carassius* sample species caught by galsama nets from Akgöl were obtained using the Laparatomy method and the dying process was performed, the samples were photographed. The obtained photographs are shown in Fig. 3. With the help of the photographs, the findings obtained by measuring the area and volumes of the nucleus of at least 100 erythrocyte cells taken from each individual with the help of photographs are given in Table 1.

Upon comparison of *C. carassius and C. gibelio* erythrocyte area, nuclear area, erythrocyte volume and nuclear volume values, it has been observed that the values of *C. gibelio* are higher than *C. carassius*. In all of the measurements it has been observed that the proportion of C. gibelio *C. carassius* is 1.3 to 1.6. Consequently it has been observed that the erythrocytes and erythrocyte nucleuses of *C. gibelio* living in Akgöl are larger than *C. carassius*.

Table 1: *C. gibelio* and *C. carassius* erythrocyte size and size ratio in different samples

	Carassius gibelio (Meam±SD)					*Carassius carassius* (Meam±SD)				
Parameters	1	2	3	4	Mean	1	2	3	Mean	Size ratio
EA (μm²)	51.9±5.5	48.7±6.7	48.3±5.5	46.2±6.7	48.8	34.3±4.8	38.8±4.33	39.4±6.5	37.5	1.3
NA (μm²)	6.8±1	7.7±3.5	6.7±0.7	7.2±1.48	7.1	4.6±0.7	4.7±0.6	4.6±0.7	4.6	1.5
EV (μm³)	225.6±40	216.8±50	201.9±34	197.5±48	210.4	131.4±25	162.3±26	168.1±46	153.9	1.4
NV (μm³)	10±2.8	12.2±5.5	9.7±2.1	11.3±4.4	10.8	6.4±1.6	7±1.7	6.8±1.9	6.7	1.6

EA: Erythrocyte area, NA: Nuclear area, EV: Erythrocyte volume, NV: Nuclear volume

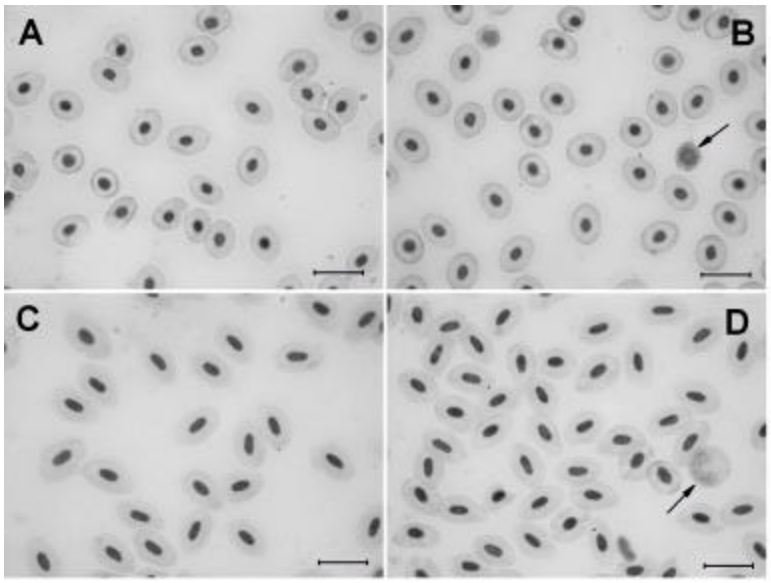

Fig. 3: Erythrocytes in blood samples of *C. carassius* (A and B, arrow lymphocyte) and *C. gibelio* (C and D, arrow monocyte), Scale bar: 10 μm

DISCUSSION

Because of the advantages particularly provided in culture fishing, the populations comprised of triploid individuals are preferred. Together with the developing technology, studies for suppression of the chromosomes related with gender formation and for creating individuals having more or larger chromosomes have been successful. Triploid fish production can be used as a biotechnological means for realization of some certain managing purposes besides the studies for increasing productivity. For instance, triploid grass carp are routinely used in biological control of aquatic vegetation since they are functionally sterile with 3 chromosome sets (Kerby and Harell, 1990).

The first inspiration sources of these methods are the species which are naturally successful in this task in the nature. Particularly *C. gibelio* can perform reproduction with gynogenesis which is not based on the male as a unique reproduction skill in the nature. In this way, different colonies can be formed in the same environment. All of the individuals reproducing with gynogenesis forms colonies comprised of females and the individuals of this colony has 156-162 chromosomes. The individuals with 2n 100 chromosomes form colonies comprised of females and males. Since the

erythrocyte area, nuclear area, erythrocyte volume and nuclear volume of the individuals with 3n 156-162 chromosomes carry more chromosomes in nucleuses, they are expected to be 50% larger than the individuals with 2n = 100 chromosomes in average. In this study, since such a difference is not observed among the erythrocyte and nucleus sizes of 4 *C. gibelio* individual, it is not possible to mention that *C. gibelio* reproduce by gynogenesis in Akgöl. As a general rule, despite it is accepted that the erythrocyte and nucleus sizes of a certain species is constant, today this rule is not so valid for the fish in particular, since the environmental conditions are effective in the erythrocyte and nucleus sizes (Najiah *et al*., 2008). Since the habitats where the fresh water fish live are contaminated severely, in response to these changing ecologic conditions, it is possible to observe different erythrocyte and nucleus sizes in the populations of the same species living in two different locations. The most significant factor affecting the erythrocyte and nucleus structure is O_2. Erythrocyte are highly specialized for transporting O_2 (Najiah *et al*., 2008). The erythrocyte and nucleus structures may change depending on the O_2 level in water, the movement level of the fish and even on the nutrition regime of the fish. When the O_2 level decreases, some fish increase the erythrocyte number in blood and therefore the sizes of erythrocyte decrease. Some heavy metals such as cadmium in the water may cause disruption of the shape of and changes the sizes of the erythrocytes in fish. The smooth, symmetric structure of the red blood cells disrupts when there is an increase interior cell calcium concentration and a decrease in ATP concentration. Such changes can be formed by the surrounding contaminators which pump calcium into cell like cadmium or decrease the ATP level in cell. In this way, the smooth shape of the cells is disrupted and the membrane obtains a fragmented structure and furthermore, the cell nucleus settles in the periphery and causes the cell to gain a spherical structure. Moreover, it has been demonstrated that the cell wall referred to as erythrocyte with echinocyte spicule takes a thorn like structure by directly impacting on the erythrocyte membranes of the various organic contaminators (Nikinmaa, 1992).

In their studies, Katalay and Parlak (2004) have performed accumulation trials for 24 days under laboratory conditions for determining the effect of cadmium in *G. niger* species on the erythrocyte structure. During their microscopic studies, as a result of the effect of cadmium, they have observed that some histological alterations have occurred and the number of immature and degenerated erythrocyte has increased. The nucleus inside the normal erythrocytes have changed and gained a spherical shape. It has been observed that the cell wall takes a thorn like structure and that there is an increase in hypochromic anemia, fragmental erythrocyte structure and in the number of micronuclei.

There are a few methods for defining the triploids, one of which is the size of the cells and the nucleus. The red blood cell namely the size of erythrocyte is often used.

There are two studies demonstrating the gynogenetical reproduction characteristic of *C. gibelio*. In the first study, the gynogenetic reproduction characteristics of the *C. gibelio* population in Uluabat Lake have been determined based on the chromosome number (Emiroglu *et al*., 2011). The second study for determining the gynogenetic reproduction feature in Turkey has been conducted on 2 fish provided from Istanbul Fish Market by Kalous *et al*. (2004). In this study, the average of the erythrocyte nucleus areas has been measured as 19.85 and 18.27 μm^2 and the results of the studies conducted in Czech Republic have been taken as reference. In accordance with the study in Czech Republic, the nucleus areas of 2n individuals have been determined as 10.76 μm^2 and the areas of 3n individuals have been determined as 18.5 μm^2 and based on these results, it has been determined that the *C. gibelio* population in Kayaliköy dam reproduces by gynogenesis. However,

it has been understood from the literature information that it is not correct to mention that the population demonstrates a gynogenetic reproduction characteristic depending on the erythrocyte size obtained from two different locations. In the third study, the gynogenetic reproduction characteristics of the *C. gibelio* population in Porsuk dam pond have been determined based on the erythrocyte sizes. In this study, the proportion of males to females has been determined by examining the genders of 381 *C. gibelio* individuals and the erythrocyte sizes have been determined based on the blood samples obtained from 4 female individuals. The nuclear area of 4 individuals has been measured as 8.50, 12.41, 8.54, 7.26 μm^2. Since the difference between the nuclear area is at least 1.45 as a result of the measurement, it has been determined that the *C. gibelio* population in Porsuk Dam includes 3n individuals (Emiroglu *et al*., 2010). Since, in this study, such a difference has not been observed among 4 *C. gibelio* individuals the nuclear area of the erythrocyte nucleus of which have been measures and the measured average nuclear area of 4 individuals (7.1 μm^2) is found to be smaller than two studies conducted in Turkey, it is not possible to mention that the *C. gibelio* population in Akgöl reproduce by gynogenesis. Theoretically the erythrocyte sizes in different populations of a species are accepted to be constant. The fact that the *C. gibelio* individuals in Akgöl have smaller erythrocyte sizes than those in Porsuk dam pond and in Kayaliköy dam indicates that they are exposed to a severe contamination in Akgöl.

The erythrocyte sizes of two species from an identical genus and with identical basic chromosome numbers should be close to each other. However, among the two species with equal chromosome numbers, the erythrocyte and erythrocyte nucleus area and volumes of *C. gibelio* is found out to be larger than *C. carassius*. The size difference in especially the erythrocyte nucleus area indicates that *C. gibelio* may demonstrate a higher adaptation power to ecologically hard conditions by means of selective gene application. The fact that the contamination emerged during the overflowing of Sakarya river which carries the wastes of the heaviest industrial organizations and the most intensive settlement units were carried to Akgöl and as a consequence of the contamination of the lake by the high level of manure and pesticide remnants in the drainage waters of the 15,000 m^2 field of agriculture surrounding the lake, a serious metal contamination occurs in Akgöl with organic and inorganic features. In such heavily contaminated waters, the fish fight for surviving. In August in 2010, many fish died in the lake depending on the oxygen deficiency in the lake.

CONCLUSION

In such intensively contaminated waters, it is also an expected situation that the erythrocyte sizes of *C. gibelio* and *C. carassius* are smaller than the normal size. However, the erythrocyte sizes of *C. gibelio* are approximately 1.5 folds larger than the *C. carassius* sizes. This situation indicates that in cases when the level of oxygen is low and the oxygen intake to the body decreases depending on the metal contamination, *C. gibelio* has the evolutional and genetic alterations to minimize the erythrocyte sizes less than *C. carassius*. This adaptation success indicates that *C. gibelio* species is more advantageous than the other species of the identical genus in environments which strengthens the continuance of live ecologically. We are of the opinion that the ecologic conditions of the environments where the two species live together and the genetic features of the species should be examined and the structures superior to *C. gibelio* should be found in order to provide great benefits for the biotechnological methods in the fishing applications.

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